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Previews

New Advances in the Effort against Ebola

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The unprecedented scale of the recent Ebola virus outbreak caused many to wonder whether this virus is different, and raised concerns about how to contain the outbreak. Two recent studies published in *Science* (Hoenen et al., 2015; Marzi et al., 2015) shed light on the subject and offer a new solution.

The recent Ebola virus outbreak began in December 2013. The first sequences of the responsible virus (3 complete and 15 partial genomes) were obtained in March 2014 (Baize et al., 2014), when the outbreak was limited to Guinea and involved fewer than 100 cases. The next sequence information was obtained in June, of viruses isolated from 99 patients in Sierra Leone (Gire et al., 2014). At that time, the outbreak was confined to Guinea, Sierra Leone, and Liberia and had infected an estimated 500 people. Now, Hoenen et al. (2015) describe sequences obtained from patients in Mali in October and November 2014 when the outbreak had expanded to ~16,000 cases and the virus had been introduced into Nigeria, Senegal, the United States, Spain, and the United Kingdom. Together, the three sequencing studies provide distinct snapshots of a burgeoning epidemic.

The original Baize study found that the three separate 2014 patient samples from Guinea were nearly identical to each other: just 6 single nucleotide polymorphisms were found in the 18,959 nucleotide Ebola virus genome. Overall, these sequences were ~97% identical to those isolated in prior outbreaks in the Democratic Republic of Congo (DRC) and Gabon, providing our first evidence that the strain of Ebola virus in West Africa was distinct from those previously noted in central Africa. Phylogenetic analysis suggested that the 2014 Guinean Ebola virus lineage evolved in parallel with the DRC and Gabon viruses and may have circulated undetected in West Africa for some time.

Determination of the evolutionary origin of the 2014 strain, and when it was physically introduced into West Africa, depends on an accurate understanding of the rate at which mutations in Ebola virus are fixed in nature, its "molecular clock." The initial exploratory study sampled three complete genomes, which may be too few for accurate clock estimation. The Gire study, performed a few months later, provided a larger sample size and estimated the molecular clock rate to be $\sim 1.9 \times 10^{-3}$ substitutions per site per year (~36 nucleotide differences per Ebola virus genome per year). This rate is substantially faster than previous estimates of mutation rates in Ebola virus (Biek et al., 2006; Carroll et al., 2013; Walsh et al., 2005).

Now the most recent study has compared samples collected in Mali in October through November 2014 to those reported in the Baize and Gire studies. Hoenen et al. (2015) note 9–20 nucleotide differences, suggesting that Ebola viruses collected early and later in this outbreak are 99.9% identical to each other. At the protein level, only one to two amino acid changes among three patients were noted between Mali and Sierra Leone samples, and four to six changes between Mali and Guinea samples. The similarity suggested a substitution rate of approximately 1×10^{-3} substitutions per site per year (19 nucleotides per Ebola virus genome per year), less than that suggested by the Gire study.

Thus, these different snapshots of the 2014 virus have led to estimates of the mutation rate between 1 and 2×10^{-3} (19–36 nucleotides per genome per year, or 0.1%–0.2% of the genome differing per year). These differences have led to contrasting headlines in the popular press. Note that these studies were performed on different sets of sequences, with different sample sizes, over different time points and in different geographic locations. The Gire study, which estimated the highest substitution rate, analyzed data from multiple time points within individual patients. This analysis may have been more likely to identify transient deleterious mutations, which are only likely to circulate for a short time period and may not become fixed in populations.

Is understanding the precise value of the clock important? An accurate representation of evolutionary rates could help determine when Ebola virus was introduced into West Africa and link that introduction to ecological factors in play at the time. Understanding breeding cycles, migration patterns, human behavior, or weather events that led to establishment of the virus in West Africa could help us predict future movement of Ebola or many of the other human pathogenic viruses carried by similar reservoir species.

A more pragmatic view is that these debated differences in molecular clock are all within the range of what has been previously noted for other RNA viruses (Duffy et al., 2008) such that they can all be thought of as rapidly evolving. Further, all 2014 sequences from West Africa in these three different studies are >99.9% identical at the nucleotide level. However, this raises another question. Given that RNA virus replication is error-prone, why do we not see more phenotypic variation among Ebola viruses? Why is the 1976 virus seemingly so similar to that from 2014? One hypothesis is that Ebola virus is not subject to much selective pressure/immune surveillance in its natural reservoir. Another hypothesis is that it is instead subject to very strong selective pressure in its reservoir, but that pressure drives purifying selection (of very little change), rather than positive selection (change in a certain direction).

On the protein level, a few dozen mutations are noted between this West African strain (now termed Makona) and Central African Ebola variants. Many of these mutations occur in regions of the proteome thought to be structurally flexible and/or previously known to be variable in sequence. One mutation could certainly alter virulence, but the effect of any of these mutations can only be determined by functional assays. Purifying selection may also operate on the protein level of the virus itself. Ebola virus only has seven genes. Each of its gene products, except perhaps secreted versions of the glycoprotein, are essential, and many are multifunctional. One protein, VP40, assumes at least three different assemblies for three distinct and essential functions. The requirement for that single polypeptide to adopt three unique tertiary and quaternary structures means that it may encode comparatively fewer amino acids tolerant of mutation.

For additional perspective, one may also note that the scale of variation among sequences of Ebola viruses is dwarfed, by orders of magnitude, by the scale of variation among sequences of HIV-1 and hepatitis C. Ebola virus causes acute infection and is cleared, or the patient perishes, within weeks. By contrast, HIV-1 and hepatitis C cause chronic infections that persist in humans for decades and are constantly subject to error-prone replication and selective immune pressure from the host. The extreme challenges in developing immunogens for HIV-1 that accurately reflect the array of circulating HIV-1 sequences likely do not apply to Ebola virus.

For public health, the key question is how to generate a vaccine to protect against Ebola virus. Fortunately, multiple candidate vaccines have shown promise in non-human primate models of infection. Many of these vaccines function by displaying the Ebola virus surface glycoprotein (GP) on carrier virus particles that differ in shape, presentation of GP, and ability to replicate. Such differences can lead to different immune responses and correlates of protection by each vaccine. In one vaccine in clinical trials, the carrier is replication-competent vesicular stomatitis virus (VSV). In another vaccine candidate, Ebola virus GP is displayed on a chimpanzee adenovirus. The adenovirus shot can be followed by boost of Ebola GP displayed on a vaccinia virus-like particle.

In a recent paper by Marzi et al. (2015), a new strategy was described in which Ebola GP is displayed on a natural Ebola virus particle that has been engineered to be replication defective. A chief difference between this vaccine and others is that this one is a (nearly) whole virus vaccine: the presence of the other viral proteins and viral nucleic acid could trigger a broader and more robust immune response than vaccination with GP as the only viral antigen. Further, this particle mimics the shape, GP display, and spacing of natural Ebola virus with fidelity.

How was this particle made replication defective? The genome of natural Ebola virus encodes a protein, VP30, which is an essential transcription factor. This vulnerability was exploited to create a version of the virus suitable for use outside of a BSL-4 containment facility (Halfmann et al., 2008). Ebola virus deleted for the VP30 gene can be replicated only in cells engineered to stably express the VP30 protein but will not replicate in any other cell, such as those of the researcher, for example. As the ΔVP30 genome is incorporated into filovirus particles that are capable of entering host cells, this system has been used as a laboratory model for functional analysis of the ebolavirus life cycle in vitro. In vivo, immunization of mice and guinea pigs with ΔVP30 particles caused no illness and protected them from lethal doses of Ebola virus (Halfmann et al., 2009). This year, the team demonstrated that vaccination with ΔVP30 also protected nonhuman primates from lethal Ebola virus challenge (Marzi et al., 2015). The vaccine elicited both T cell and antibody responses, and because this is a (nearly) whole-virus vaccine, antibodies were elicited against the surface GP as well as the internal nucleoprotein NP and matrix protein VP40.

Immunization of a particle that so closely resembles the natural virus may raise eyebrows, although in animal studies this particle has not altered its behavior or acquired the missing gene. Whole-virus vaccines (either inactivated or attenuated) have previously been used in humans against influenza, smallpox, measles, and mumps. Note that Ebola virus has no integrase in its lifecycle and has no segments to reassort, as does influenza. However, to alleviate the possibility of something unexpected happening, the team sought to inactivate the ΔVP30 particles so that they are replication defective whether or not VP30 is present. They found that hydrogen peroxide treatment of the ΔVP30 particles inactivates them but retains their immunogenicity (Marzi et al., 2015). An interesting and potentially useful feature of this vaccine strategy is that it involves a filovirus particle. Hence, vaccine recipients probably won't have prior immunity against the carrier virus itself, which can prevent the desired immune responses. Use of a filovirus particle does not "use up" that particle carrier in a vaccinee's potential immune portfolio: many of the same carrier viruses are being considered for vaccines against different viruses.

Multiple types of Ebola virus vaccines have now been shown to work well in nonhuman primate models. The next questions are: will they work in humans? How long will immunity last? If Ebola virus breaks out again in 10 years, will vaccinees still be immune? Only time will tell.

The wealth of information and technology moving forward offers hope that we will not have another crisis quite like 2014—for this virus, anyway. We are, however, much less prepared for other filoviruses and co-circulating pathogens. Surveillance, vigilance, and maintaining progress are key.

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